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Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on the HPV test plan for 6-tert-butyl-3-chloromethyl)-2,4-xylenol (A-1846).

Dear Administrator Leavitt:

The following are comments on the HPV test plan for A-1846 (CAS no. 23500-79-0), submitted by Cytec Industries, Inc. These comments are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health and environmental protection organizations have a combined membership of more than ten million Americans.

Cytec proposes conducting an acute fish toxicity test (OECD no. 203), a mammalian 28-day oral toxicity test (OECD no. 407), and a mammalian developmental toxicity test (OECD no. 414). These tests will kill at least 1,460 animals.

With respect to the mammalian studies, the 28-day test is clearly inappropriate. Cytec demonstrates that A-1846 is a closed-system intermediate (pp. 13-14), and correctly concludes that no reproductive toxicity testing is necessary (p. 10). However, according to the EPA, subchronic tests on closed system intermediates are equally inappropriate, as stated in the October 1999 EPA letter to HPV participants: "Participants shall not develop sub-chronic or reproductive toxicity data for the HPV chemicals that are solely closed system intermediates" (Wayland 1999).

This requirement was reiterated in December 26, 2000 *Federal Register* notice entitled "Data Collection and Development on HPV Chemicals": "One principle is that participants **shall not** [emphasis added] develop sub-chronic or reproductive toxicity data for the HPV chemicals that are solely closed system intermediates" (p. 81689).

With regard to the proposed developmental toxicity test, there is no justification whatsoever for conducting the proposed OECD no. 414 which kills at least 1,300 animals. This is a screening level program and even the EPA has encouraged companies to use either OECD 421 or 422 which kill approximately half the number of animals than the 414. Based on previous correspondence with Cytec on other test plans, we are hopeful that the proposal to conduct the 414 is simply an oversight that will be corrected. Further, given that this substance is a closed system intermediate, it

would in fact be more appropriate for Cytec to conduct the *in vitro* rodent embryonic stem cell test *prior to* making any decisions regarding the need for the *in vivo* developmental toxicity test (Genschow 2002).

We are also concerned that Cytec does not appear to have attempted to predict the substance's developmental toxicity from its structural relationship with other halogenated alkyl phenols, especially as substituted phenols are the category of compounds with which the most extensive comparison of *in vivo* and *in vitro* structure-activity relationships has been carried out (Kavlock 1992). The EPA has clearly stated that "Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships" (Wayland 1999, EPA, *Federal Register* 2000).

We note that Cytec plans to carry out an *in vitro* chromosomal aberration assay (OECD 473; p. 10). This assay is most commonly carried out using Chinese hamster ovary cells. However, human lymphocytes can be used equally readily, and we hope that Cytec will avail itself of this option.

Lastly, the fish toxicity test is clearly inappropriate, because the octanol/water partition coefficient is appears too high. The EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and it recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead (EPA, *Federal Register* 2000, pp. 81679, 81695). The log $K_{o/w}$ value of A-1846 has been calculated to be 5.32 (test plan, p. 6). The value has also been measured and found to be 3.9, just below the EPA's limit for fish tests, but that was in the form in which it is usually stored, containing 11-13% methyl isobutyl ketone. We do not understand why Cytec does not intend to measure the log $K_{o/w}$ value of A-1846 as part of the test plan, and it is highly premature to carry out a fish test without performing such measurements. Furthermore, if Cytec wishes to investigate the acute fish toxicity of A-1846, we urge it to use one or more of the several available *in vitro* methods (see Appendix). In addition, acute fish toxicity data have already been obtained (p. 8) using an *in silico* method, ECOSAR, which is recommended by the EPA (EPA 2002), yet Cytec does not provide an explanation as to why it does not accord any weight to these results.

Thank you for your attention to these comments. We would greatly appreciate receiving a response to these issues. I can be reached at 202-686-2210, ext 335 or by email at *kstoick@pcrm.org*.

Sincerely,

Kristie Stoick, M.P.H.
Research Analyst

Appendix: *In vitro* alternatives to the acute fish toxicity test

TETRATOX, an assay based on the *protozoan Tetrahymena pyriformis* (Larsen 1997), is the most appropriate *in vitro* method. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After almost two years, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

The recently validated *DarT* test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *DarT* test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *DarT* test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *DarT* test were highly concordant with in vivo reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

References

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